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Investigation 4: Diffusion and Osmosis

Background

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins; the phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solutes (e.g., salts, organic molecules) dissolve in water, which is the solvent. Water may pass slowly through the membrane by osmosis or through specialized protein channels called aquaporins. Aquaporins allow the water to move more quickly than it would through osmosis. Most other substances, such as ions, move through protein channels, while larger molecules, including carbohydrates, move through transport proteins.

The simplest form of movement is diffusion, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input by cells. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by diffusion; the movement of water through membranes is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high free water concentration) and low solute concentration to areas of low potential (low free water concentration) and high solute concentration. Solutes decrease the concentration of free water, since water molecules surround the solute molecules. The terms hypertonic, hypotonic, and isotonic are used to describe solutions separated by selectively permeable membranes. A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solution through the membrane by osmosis. A hypotonic solution has a lower solute concentration and a higher water potential than the solution on the other side of the membrane; water will move down its concentration gradient into the other solution. Isotonic solutions have equal water potentials.

In nonwalled cells, such as animal cells, the movement of water into and out of a cell is affected by the relative solute concentration on either side of the plasma membrane. As water moves out of the cell, the cell shrinks; if water moves into the cell, it swells and may eventually burst. In walled cells, including fungal and plant cells, osmosis is affected not only by the solute concentration, but also by the resistance to water movement in the cell by the cell wall. This resistance is called turgor pressure. The presence of a cell wall prevents the cells from bursting as water enters; however, pressure builds up inside the cell and affects the rate of osmosis. Water movement in plants is important in water transport from the roots into the shoots and leaves.

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Understanding Water Potential

Water potential predicts which way water diffuses through plant tissues and is abbreviated by the Greek letter psi (ψ). Water potential is the free energy per mole of water and is calculated from two major components:

(1) the solute potential (ψS), which is dependent on solute concentration, and

(2) the pressure potential (ψP), which results from the exertion of pressure—either positive or negative (tension) — on a solution.

Note: The solute potential is also called the osmotic potential.

$$\psi = \psi P + \psi S$$

Water Potential = Pressure Potential + Solute Potential

Water moves from an area of higher water potential or higher free energy to an area of lower water potential or lower free energy. Water potential measures the tendency of water to diffuse from one compartment to another compartment.

The water potential of pure water in an open beaker is zero ($\psi = 0$) because both the solute and pressure potentials are zero ($\psi S = 0$; $\psi P = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water lowers the solute potential and therefore decreases the water potential. This means that a solution at atmospheric pressure has a negative water potential due to the solute.

The solute potential (ψS) = – iCRT, where i is the ionization constant, C is the molar concentration (a.k.a. osmolarity), R is the pressure constant (R = 0.0831 liter bars/mole-K), and T is the temperature in K (273 + °C).

A 0.15 M solution of sucrose at atmospheric pressure ($\psi P = 0$) and 25°C has an osmotic potential of -3.7 bars and a water potential of -3.7 bars. A bar is a metric measure of pressure and is the same as 1 atmosphere at sea level. A 0.15 M NaCl solution contains 2 ions, Na+and Cl-; therefore i = 2 and the water potential = -7.4 bars.

When a cell's cytoplasm is separated from pure water by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher ($\psi = 0$), into the cell, where water potential is lower because of solutes in the cytoplasm (ψ is negative). It is assumed that the solute is not diffusing (Figure 1a). The movement of water into the cell causes the cell to swell, and the cell membrane pushes against the cell wall to produce an increase in pressure. This pressure, which counteracts the diffusion of water into the cell, is called turgor pressure.

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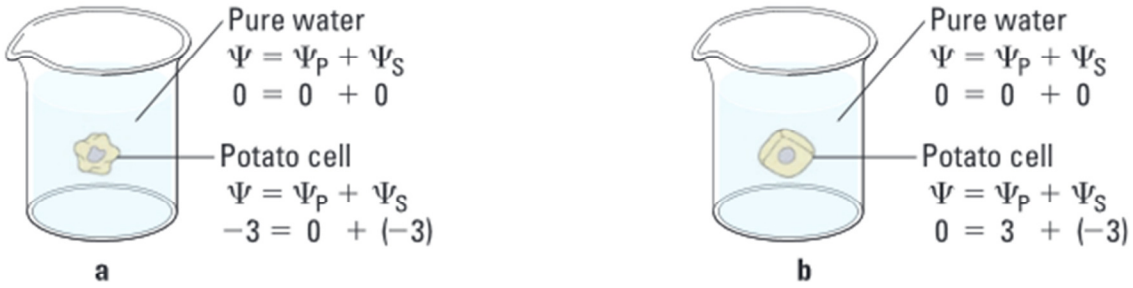
Over time, enough positive turgor pressure builds up to oppose the more negative solute potential of the cell. Eventually, the water potential of the cell equals the water potential of the pure water outside the cell (ψ of cell = ψ of pure water = 0). At this point, a dynamic equilibrium is reached and net water movement ceases (Figure 1b).



Figures 1a-b. Plant cell in pure water. The water potential was calculated at the beginning of the experiment (a) and after water movement reached dynamic equilibrium and the net water movement was zero (b).

If solute is added to the water surrounding the plant cell, the water potential of the solution surrounding the cell decreases. If enough solute is added, the water potential outside the cell is equal to the water potential inside the cell, and there will be no net movement of water. However, the solute concentrations inside and outside the cell are not equal, because the water potential inside the cell results from the combination of both the turgor pressure (ψP) and the solute pressure (ψS). (See Figure 2.)

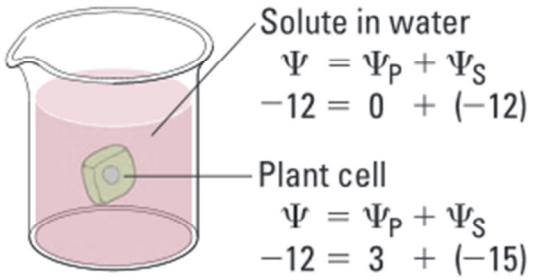


Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not equal.

If more solute is added to the water surrounding the cell, water will leave the cell, moving from an area of higher water potential to an area of lower water potential. The water loss causes the cell to lose turgor. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

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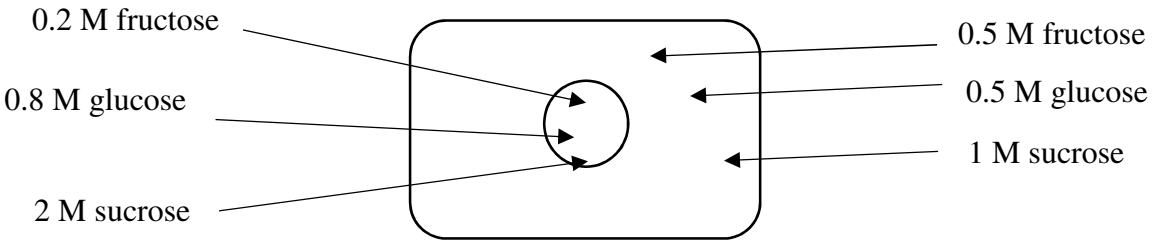
Pre-Lab Questions

1. Consider the following scenario: An artificial membrane that is selectively permeable encloses an aqueous solution. The solution surrounding the model cell contains a different aqueous solution. Assume that monosaccharides such as glucose and fructose are able to cross the membrane but that larger disaccharides, such as sucrose, do not. The exact concentrations of each solute in the cell and surroundings are shown in the figure below.



   a. Which solute(s) will exhibit a net diffusion into the cell?

   b. Which solutes(s) will exhibit a net diffusion out of the cell?

   c. With respect to glucose, is the surrounding environment hypertonic or hypotonic to the cell?

2. Given the following solutions in the model cells vs surrounding environments, predict whether there will be net diffusion of water by osmosis into or out of each model cell. Enter your predictions in the table below.

#	Model Cell (inside dialysis tubing)	Surrounding Environment (plastic cup)	Net Diffusion (into or out of cell)
1	1M NaCl	Water	
2	Water	1M Glucose	
3	1M Sucrose	Water	
4	Water	Water	

3. Calculate the solute potential of a 0.1M NaCl solution at 25ºC. SHOW YOUR WORK!

4. If the concentrations of NaCl inside a plant cell is 0.15M, which way will the water diffuse if the cell is placed into the 0.1M NaCl solution?

5. What must the turgor pressure equal if there is no net diffusion between the above solution and cell? SHOW YOUR WORK!

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Part I

Procedure

1. Obtain four plastic cups and label them 1-4.
2. Obtain four 18-cm pieces of pre-soaked dialysis tubing.
3. Twist approximately 3cm of tubing at one end and tie into a knot.
4. Using a graduated cylinder, measure 15mL of the specified solution listed for Model Cell #1 (found in Table 1 in the results section).
5. Open the opposite end of the dialysis tubing by rubbing it together between your fingertips.
6. Place a funnel in the open end of the dialysis tubing and transfer the 15mL of solution into the model cell.
7. Twist and knot the open end of the tubing to seal the bag of dialysis tubing, leaving enough space for water to diffuse into the cell.
8. Gently rinse the dialysis tube bag with DI water to make sure none of the solution with the bag has dripped on the outside.
9. Place the dialysis tube bag on a paper towel and gently roll it back and forth to remove any excess liquid.
10. Measure and record the mass of the dialysis tube.
11. Fill cup #1 with 100mL of the specified solution listed in the Surrounding Environment #1 (found in Table 1 in the results section).
12. Place the dialysis tube bag in cup 1.
13. Repeat steps 3-12 with the remaining solutions.
14. The next day, remove the dialysis tube bag from cup #1. Gently roll it back and forth on a paper towel to remove excess liquid. Measure and record the final mass of the dialysis tube bag. Repeat for the remaining cells/cups.
15. For each model cell, calculate the percent change in mass and record in Table 1. Be sure to indicate if the % change in mass is positive or negative!

$$\text{Percent Change} = \frac{(\text{Final Mass} - \text{Initial Mass})}{\text{Initial Mass}} \times 100\%$$

Results

Table 1

#	Model Cell (inside dialysis tubing)	Surrounding Environment (plastic cup)	Initial Mass	Final Mass	% Change in Mass
1	1M NaCl	Water			
2	Water	1M Glucose			
3	1M Sucrose	Water			
4	Water	Water			

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Analysis and Applications

1. Which setup (#) had the least change in mass? How can you explain this?

2. Write the number of the setup(s) in which the environment(s) was/were considered
   - Hypertonic
   - Hypotonic
   - Isotonic

3. Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?

4. What might happen to the plants in a freshwater wetland if saltwater from an estuary or the ocean suddenly flooded it? Explain your answer.

You are in the hospital and need intravenous fluids. You read the label on the IV bag, which lists all of the solutes in the water.

5. Why is it important for an IV solution to have salts in it?

6. What would happen if you were given pure water in an IV?

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Part II

Procedure

Help! I prepared solutions of 1M, 0.8M, 0.6M, 0.4M, 0.2M, and 0M sucrose solutions, but forgot to label them. After realizing my error, I randomly labeled the flasks containing these 6 unknown solutions as A-F and need your help figuring out which is which so we can do the next part of the lab. Given what you learned in Part I of the lab, design an experiment to identify the concentrations of the sucrose solutions in the space below.

Results

Table 2

Solution	Initial Mass	Final Mass	% Change in Mass	Sucrose Concentration
A				
B				
C				
D				
E				
F				

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Part III

Procedure

1. Label 6 test tubes with the 6 different sucrose solutions identified below in Table 3.
2. Use a cork borer to cut 12 potato cylinders. Trim the cylinders so they are all the same length, removing any skin found on the cylinders.
3. Determine the mass of 2 of the cylinders together and record in Table 3. Put these 2 cylinders into the appropriately labeled sucrose solution test tube.
4. Repeat for the remaining test tubes/solutions, recording masses of 2 potato cylinders at a time.
5. Fill each tube with the appropriate sucrose solution. Be sure the potato cylinders are complete covered. Each test tube should have about the same volume of solution.
6. Cover the test tubes and let stand overnight.
7. The next day, record the temperature of the sucrose solutions here: _____ºC
8. Remove the cores from one of the test tubes, blotting them gently with a paper towel to remove excess water (but don't soak up water from inside the cylinder), and record their combined mass in Table 3.
9. Repeat for the other test tubes.
10. Calculate their percent change in mass and record in Table 3.

Results

Table 3

Contents of Test Tube	Initial Mass	Final Mass	% Change in Mass
0.0M sucrose			
0.2M sucrose			
0.4M sucrose			
0.6M sucrose			
0.8M sucrose			
1.0M sucrose			

Analysis

Explain at least 2 possible sources of error in your procedure/data collection that could affect the results in this lab (they can be from both/either Part II and/or III):

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-

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Using the hints below, determine the

- Molar concentration (or osmolarity) of the potatoes _____
    - You must have a graph to support your findings!
- The osmotic (or solute) potential of the potatoes _____
    - Show your work for this calculation here:

- Determining Osmolarity
    - Graph the percent change in mass vs molar concentration of sucrose
        - Give your graph a title that is descriptive of the subject matter being graphed
        - Label your axes (include units) and number your scales with equal intervals
            - Molar concentration of sucrose is your independent variable and should be plotted on the x-axis
            - % change in mass is your dependent variable and should be plotted on the y-axis
        - Note: Compare to the potato cores
            - A positive % change in mass indicates a hypotonic environment
            - A negative % change in mass indicates a hypertonic environment
        - Draw a best-fit line
            - The point at which this line crosses the x-axis indicates an isotonic environment, as the mass of the potato cores did not change
            - In other words, the molar concentration of sucrose at this point has a water potential equal to the potato tissue water potential
            - Thus the molar concentration of sucrose at this point is equal to the molar concentration (or osmolarity) of your potato!

- Determining Osmotic Potential
    - Remember, the solute potential (ψ_S) = – iCRT,
        - i = 1 (the ionization constant for sucrose)
        - C = osmolarity of potato (which you just determined from your graph!)
        - R = 0.0831 liter bars/mole-K (pressure constant)
        - T = temperature in K (273 + °C)